

SOUTHERN BLOTTING

INTRODUCTION:

E. M. Southern (1975) first described a method for the transfer of DNA restriction fragments from agarose gels to cellulose nitrate (nitrocellulose) membranes and then hybridization of the resulting blot-immobilized DNA to radioactively labeled DNA probes. Since then, the term "Southern blot" has been used to describe any hybridization of DNA on blots to DNA probes, regardless of the type of membrane used. [Northern blot refers to RNA/DNA blot hybridizations, Western blots are protein/antibody hybridizations and other "directional" blotting terms have also been used.]

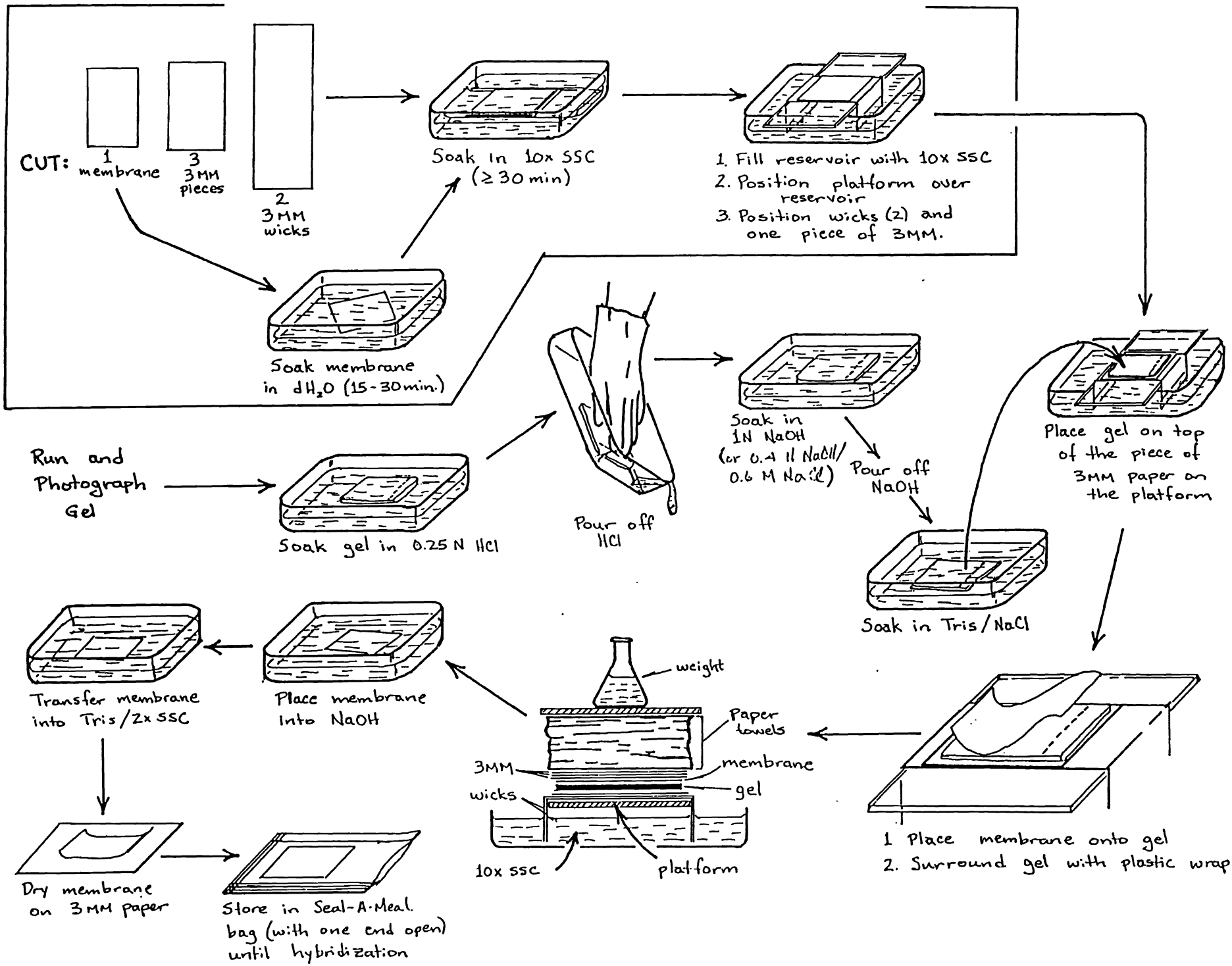
The original and subsequent methods are based on capillary movement of the solution (usually a version of a solution containing sodium chloride / sodium citrate or sodium hydroxide) which carries the DNA with it. The solution is able to travel through gel and the membrane, while the DNA is carried out of the gel and becomes bound to the membrane. Because the membranes are more reactive towards single stranded DNA than double stranded, the DNA must first be denatured by treatment with base (most often sodium hydroxide).

Recently, other methods have been developed for the transfer of the DNA to the membranes. Several vacuum blotting systems are commercially available. They speed up the blotting process a great deal, but do not seem to produce any better results than do the standard capillary action blotting methods. Electroblothing methods have also been developed, but they work best for proteins and in many cases produce unsatisfactory results with nucleic acids.

Below is described the method for transfer of the DNA (or RNA) nylon-based membranes (Gene Screen Plus, Nytran, Zetabind, etc.) using the capillary blotting (Southern, 1975) method.

STEPS IN THE PROCEDURE:

1. Run and photograph the gel.
2. Cut one piece of blotting membrane to fit the gel. NEVER HANDLE MEMBRANE WITH BARE HANDS! Use gloved hands or forceps to handle the membrane at all times. Wet the membrane for at least 15 minutes in distilled water.
3. Cut four pieces of 3MM chromatography paper which are about a centimeter longer and wider than the gel (and blot). [Always handle the 3MM paper with gloved hands. DO NOT REACH INTO THE 3MM PAPER BOX WITH BARE HANDS!!] Cut two pieces of 3MM paper that are as wide as the other 3MM pieces but are long enough to span the gel platform and have each end reach the bottom of the solution reservoir. These two pieces are called the "wicks." Place all of these pieces into the 10X SSC solution. When the blot has soaked sufficiently in the distilled water, also place it into the 10X SSC.
4. For pulse-field gels (CHEF) or other gels which have very large DNA fragments, UV treatment to nick the DNA may also be necessary. This consists of putting the gel one foot under a UV source (germicidal lamp or other type that has an output at around 254 nm) for 5-10 minutes).



[For some large gels you may also want to trim the gel to fit the autoradiography film size. In this case, the wells and 3-6 cm of the top of the gel may be trimmed off before blotting.]

5. Immerse the gel in 0.25N HCl for 15-30 minutes. [This causes nicks in the DNA.]

6. Transfer the gel (in a tray or, for larger gels, with a scoop) into a solution of 0.4N NaOH / 0.6M NaCl for 30 minutes, with agitation. [This will denature the DNA, so the two strands will come apart.]

7. Transfer the gel into a solution of 0.5M Tris (pH 7.5) / 1.5M NaCl for 30 minutes, with agitation. [This will neutralize the gel and DNA. This step can be eliminated if transfer is to be accomplished using NaOH and not 10X SSC.]

8. Set up the blots as follows:

- a. Place the 10X SSC into the reservoir (glass baking dish or other container).
 - b. Place a piece of plastic (that will span the reservoir) over the reservoir.
 - c. Place the two wicks across the plastic and push the ends down into the reservoir.
 - d. Place one piece of 3MM paper (the pieces that are slightly larger than the gel) **centered** on top of the wicks.
 - e. Carefully position the gel (top side up) on top of the piece of 3MM paper.
 - f. **Carefully** place the blotting membrane on top of the gel. Once it has touched the gel try to avoid much repositioning of the membrane as much as possible. [REMEMBER: HANDLE THE BLOTTING MEMBRANE AND 3MM PAPER WITH GLOVED HANDS ONLY, otherwise your final product will have fingerprints and blotches which will probably obscure the bands of interest.]
 - g. Place plastic wrap around all four edges of the gel.
 - h. Place the three remaining pieces of 3MM paper on top of the membrane.
 - i. Place a stack (1-2 inches thick) of paper towels (cut the same size as the 3MM papers) and place a weight on top of the paper towels. The weight should be sufficient to put pressure on the paper on the gel and blot, but not so much as to squash the gel. For a small blot, a platform with a flask on top filled with 100-200 ml of water is an acceptable weight. Be sure that the weight is evenly distributed over the paper towels.
 - j. Allow the blotting to proceed for 6-12 hours.
9. Remove the weight and the paper towels. [Save the towels that are not wet, throw the others away.]

10. **Carefully** peel back the 3MM papers and the blotting membrane. Remove the blotting membrane with forceps and/or gloved hands. When you touch the membrane with forceps or gloved hands, handle it in places that do not have DNA (i.e., the top or sides are the safest places). Place the membrane immediately into a solution of 0.4N NaOH for 30 seconds to denature any remaining double stranded pieces of DNA.
11. Transfer the membrane into 0.2M Tris (pH 7.5) / 2X SSC for 30 seconds.
12. Place the membrane onto a clean dry sheet of 3MM paper. Cross-linking of the DNA with a 3 minute exposure to UV is optional. Dry the membrane in a drying oven for 30 minutes or allow the membrane to dry for several hours at room temperature. For safe keeping until the hybridization is performed, slide the membrane into a Seal-A-Meal bag (using the 3MM paper to aid in sliding the flimsy membrane into the bag) and seal one end. When prepared for the prehybridization/hybridization, the blot will be ready.

SOLUTIONS:

0.25 N HCl (per liter)

21.5 ml concentrated HCl

0.4 N NaOH / 0.6 M NaCl (per liter)

16.0 g NaOH

35.1 g NaCl

0.5 M Tris (pH 7.5) / 1.5 M NaCl (per liter)

63.5 g Tris HCl

11.8 g Tris base

87.7 g NaCl

20X SSC (3.0 M Sodium chloride - 0.3 M sodium citrate)

175.3 g NaCl

88.2 g sodium citrate

adjust pH to 7.0 with 10N NaOH before bringing
to final volume of 1 liter